REMARKS

Claims 1-2, 4-5, 50-72 are pending and subject to examination. Claims 66-69 further narrow the scope of claim 1, and are supported, e.g., by isolates 05-D02, 05-D05, 05-G06, and 05-H09, in Table 4 of the specification, respectively. Support for claim 56 can be found, e.g., at page 9, line 7.

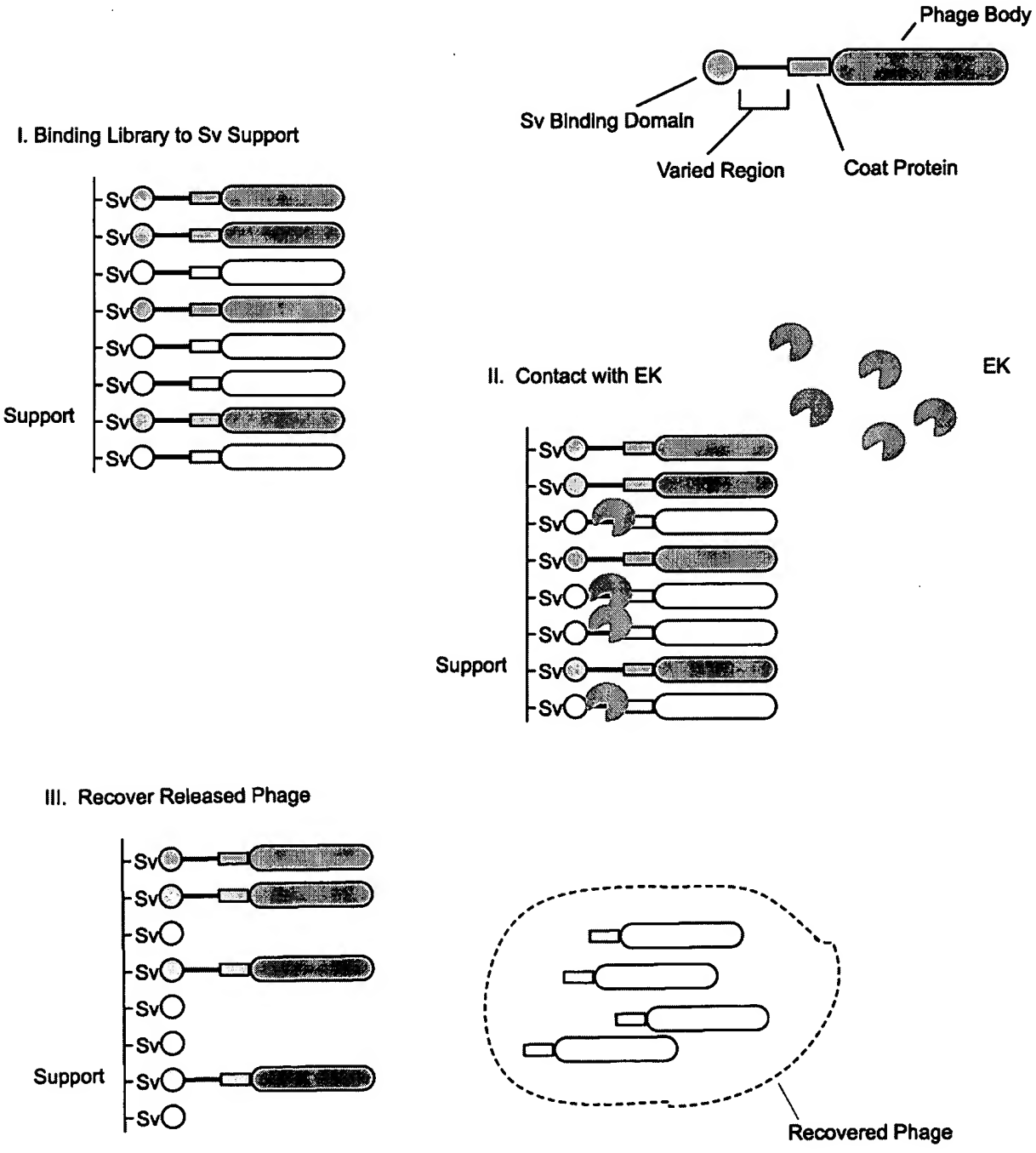
The exemplary display library screen described in the specification indicates that the Applicants have identified about 90 isolates that have amino acid sequences cleavable by enterokinase. The design of the display library screen is such that library members isolated from the screen encode proteins that were, in fact, cleaved by enterokinase. The following is a brief overview of the exemplary display library screening process and refers to a diagram on the next page:

First, a display library was prepared. A typical library member is diagrammed in the upper right corner. Each member includes a phage body that has a nucleic acid encoding a protein that is displayed on the phage surface. The encoded protein includes a streptavidin (Sv) binding domain, a varied region of 13 amino acids, and a coat protein.

Members of the library were immobilized on a solid support that includes streptavidin (shown as I, upper left). Members of the library that include a varied region that is not cleaved by enterokinase are shaded whereas members that include a varied region that is cleaved by enterokinase are not shaded. Because all members include a streptavidin binding domain, initially they are all bound to the support.

After binding, the support was treated with enterokinase. This step is shown in the middle figure as II. Enterokinase only cleaves varied regions that have a site that it can recognize and cleave. Members that do not include such a region (shaded) are not cleaved. The members that do include such a region (shown as unshaded) are cleaved, and the streptavidin binding domain is severed from the phage body. Hence, phage with cleavable varied regions are released from the support. In the final step, the buffer surrounding the support is removed and the released phage library members are recovered (bottom panel). Sequencing of the nucleic

acid in the recovered phage library members reveals the amino acid sequence of the varied regions **that were, in fact, cleaved by enterokinase**.



Applicant's remarks are preceded by quotation of the Examiner's comments from the most recent action in small bold face type.

> **Claims 1-2, 4-5 and 50-51 are rejected under 35 U. S. C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention and in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. This is a combination written description and enablement rejection. [emphasis added]**

The written description requirement is separate and distinct from the enablement requirement. MPEP § 2161. The Applicants respectfully request that the Examiner separately present the written description and enablement rejections. In the event that an appeal is lodge for this application, a complete understanding of the legal basis for the Examiner's rejection would facilitate a thorough analysis of the issues.

The Applicants infer that the following remarks of the Examiner are an enablement rejection:

> **As discussed in the previous rejection, there is not seen to be justification for the general formula of claim 1 in the instant specification. The specification states on page 44 that "an amino acid was regarded as preferred at a given position in the sequence if it occurred in five or more isolates". It is not seen that applicants have justification for arbitrarily picking 5 occurrences to make a general formula. If applicants are to put forward a general rule they should have some justification for it other than an arbitrarily picked number of 5 occurrences using the specific conditions in the examples. In the amendment filed 11/20/03, applicants argue that, as discussed in the specification on pages 21-22, a "complete random library to be scanned for enterokinase cleavage activity was designed to allow for any amino acid except for cysteine to occur at each position...[and that] in such a random library, the likelihood of a particular amino acid being present at a particular position... is... 5.2%". In their screening assay, "Applicants obtained 90 isolates...[and if an amino acid was present at a particular position in 5 or more of the 90 isolates, this is greater that a 5.2%chance of the amino acid occurring at that position randomly...[and indicates] that such an amino acid may play a role in cleavage by an enterokinase".**

The Examiner alleges that the Applicants lack a "justification for arbitrarily picking 5 occurrences to make a general formula." Regardless of the merits of this assertion, it is not, *as a matter of law*, grounds for finding lack of enablement. In Fromson v. Advance Offset Plate, Inc.,

the Federal Circuit stated, "**it is axiomatic that an inventor need not comprehend the scientific principles on which the practical effectiveness of his invention rests.**" 720 F.2d 1565, 1570 (Fed. Cir. 1983); see also, In re Cortright, 165 F.3d 1353, 1359 (quoting Fromson for this proposition). Here, the Examiner has not alleged facts which indicate that the claimed fusion protein would not be cleaved by enterokinase, rather the Examiner asserts an apparent lack of explanation, i.e., scientific principle, for the claimed protein.

Moreover, the specification describes (e.g., in Table 4) numerous peptides that are cleaved by enterokinase. Isolates 05-H04, 05-D02, 05-D05, 05-G06, and 05-H09, for example, are all peptides that were cleaved by enterokinase during the phage selection process described above. Accordingly, the Applicants respectfully submit that the Examiner's first grounds for the enablement rejection should be withdrawn.

Regarding the length of the protease recognition site (an issue separate from the frequency of an amino acid at a given position, the Examiner stated:

> **The teaching in the paragraph referred to spanning pages 21-22 is that the region tested "consisted of 13 consecutive amino acids". Pending claims 1, 2, 4-5 and 50-51 contains only 7 amino acids, not 13, and therefore it is not seen how the discussion of pages 21-22 enables one to include the amino acids found 5 or more times in the general formulae of the instant claims. There were nearly twice as many amino acids in the phage library tested as are present in the instant claims and these additional amino acids may well have some effect on the percentage occurrence of a particular amino acid at a particular position. The examiner stated in the advisory action mailed 12/11/03 that this argument "may have merit, but it was not further considered because applicant has not overcome the 112 first paragraph rejection". The argument has now been considered.**

The specification does teach that the reason to be varied in one particular example was thirteen amino acids. However, **the size of the varied region does not dictate the number of amino acids recognized by enterokinase**. Moreover, since each position is varied independently, the size of the varied region does not impact the expected frequency due to chance alone at an adjacent position.

The specification teaches that enterokinase cleaves short peptide sequences, for example, sequences "5 or 6 amino acids long" (page 4, line 20 of the specification). Moreover, line 1 of

Table 8 in the specification teaches that enterokinase cleaves a hexapeptide (GDDDDK-β-naphthylamine) at a rate of 0.46 nmole/min. In view of this data, it cannot be questioned that enterokinase can cleave a substrate as small as six amino acids.

Although the library used in the specification contained 13 varied positions, analysis of the identified sequences allowed locating the scissile bond and characterization of the hexamer sequence N-terminal to the scissile bond within the varied region. See, e.g., the paragraph beginning at page 34, line 29 of the specification. In particular, an "acidic amino acid-basic amino acid" motif identifies the relevant short peptide within the larger varied region.

Production of the peptides in Table 8 of the specification confirms that peptides much smaller than 13 amino acids in length are cleaved by enterokinase. This experiment demonstrates unequivocally that the hexameric region N-terminal of the scissile bond is sufficient for recognition by enterokinase. In Paragraph 5 of Dr. Ladner's Declaration, dated April 27, 2004, Dr. Ladner further reported that a hexameric enterokinase site (DINDDR) was successfully used to cleave a Kunitz domain from sequences N-terminal to the scissile bond.

The specification and Dr. Ladner's Declaration are unequivocal in teaching that the enterokinase can recognize a small sequence, e.g., a hexamer. These experiments confirm that the claimed region is sufficient for recognition and cleavage by enterokinase. Thus, the second grounds for the enablement rejection should also be withdrawn.

The Examiner also questioned which amino acids were allowable at the P1' position. The Applicants infer that the following comments of the Examiner are in the form of an enablement rejection:

> **In the amendment filed 11/20/03, applicants further refer to pages 43-44 which states that "only four amino acids were not observed in any of the isolates at the P1' position following Asp-Arg, among those isolates sequenced: Lys, Pro, Arg and Cys (which was not permitted in the 13-mer variable portion when the substrate phage library was generated)". They then go on to quote from these pages that "[t]he absence of any phage isolates exhibiting these amino acids at the PI position does not mean that an EK recognition sequence... having Lys, Pro, Arg or Cys at the...[P1]position will not be cleaved; rather it indicates that such recognition sequences will be cleaved less efficiently than recognition**

sequences having the other amino acids at the Xaa(P1)position" and they state that "it is clear that the Xaa5 position of the formula in claim 1 can be any amino acid". The examiner does not agree. Applicants have found absolute no instances of the Xaa5 position being Lys, Pro, Arg or Cys and it is maintained that absent convincing proof to the contrary, these residues should not be allowed at position Xaa5 in the formulae of the instant claims.

First, the absence of cysteine is not surprising since the library did not allow cysteine in the varied region. The side chain of cysteine has similar size and geometry as serine, which was among those amino acids identified at Xaa5.

Arginine is clearly tolerated at the Xaa5 position. For example, as described in Table 7 of the specification, the peptide GNYTDRRFI has arginine at the Xaa5 position. The products of cleaving this peptide were analyzed by HPLC and mass spectroscopy. The products, indeed, corresponded to cleavage products generated by cleavage N-terminal to the Xaa5 position. Lysine, like arginine, is basic and large; and is also expected to be tolerated at the Xaa5 position.

Moreover, that only four amino acids were absent from the P1' position in one particular experiment is **itself evidence that a wide range of side chain structures are tolerated at the P1' position**, in contrast to the P1 and P2 positions. The sixteen amino acids identified include, for example, glycine, which has no side chain; tryptophan, which has a large aromatic side chain; leucine, which has a large aliphatic side chain; and glutamic acid, which has a small negatively charged side chain. These observations compel the conclusion that protease specificity is not dramatically affected by the amino acid at the Xaa5 position.

Note also that new claim 61 excludes Pro from the Xaa5 position. New claim 62 excludes Pro and Cys from the Xaa5 position. New claim 63 excludes Lys, Pro, and Cys from the Xaa5 position. New claim 64 excludes Lys, Pro, Arg, and Cys from the Xaa5 position.

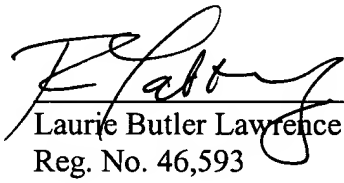
In view of the above arguments, the Applicants respectfully submit that the Examiner's third and final grounds for the (apparent) enablement rejection should also be withdrawn.

The Applicants respectfully submit that all claims are in condition for allowance, which action is expeditiously requested. The Applicants do not concede any positions of the Examiner that are not expressly addressed above. All amendments and cancellations are made without prejudice and disclaimer and may be made for reasons not explicitly stated or for reasons in addition to ones stated.

Please apply any charges for additional claims and any other charges required to deposit account 06-1050.

Respectfully submitted,

Date: 14 October 2004



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